

The University of Chicago

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I. METABOLISM STUDIES IN MAN

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III. OSTEITIS FIBROSA PRODUCED IN PUPPIES

IV. EFFECTS OF ADMINISTRATION OF IRRADIATED ERGOSTEROL

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENTS OF MEDICINE AND
PHYSIOLOGY

BY

JOSEPH L. JOHNSON, M.D.

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EXPERIMENTAL CHRONIC HYPERPARATHYROIDISM.*

I. METABOLISM STUDIES IN MAN.

BY J. L. JOHNSON,

CHICAGO, ILL.

(From the Department of Medicine, University of Chicago. The expenses of this research were met from the John D. Hertz Fund.)

SINCE the report in 1926 of Mandl's¹ case of osteitis fibrosa, in which the operative removal of a parathyroid tumor was clinically beneficial, a number of cases of bone disease have been reported for which overfunction of the parathyroid glands has been thought to be the underlying cause. These cases represent, for the most part, the generalized skeletal abnormality first clearly described by von Recklinghausen² and designated by him as osteitis fibrosa osteoplastica, but hypertrophy and tumor formation of the parathyroids have been noted also in osteomalacia and rickets, as well as in multiple myeloma and metastatic carcinoma. It thus was not quite clear whether hyperfunction of the parathyroids produced one sharply distinguishable disease or could be responsible for a variety of bone lesions and it seemed that the subject might be clarified by reproducing a chronic state of hyperparathyroidism in otherwise normal animals, and observing histologically and otherwise, the type of skeletal deformity which would result.

Our interest in the problem was aroused by personal experience with 2 cases of bone disease, in both of which parathyroid tumors were found. Further stimulus was given to our interest by the extensive metabolism studies of Aub and his associates^{3, 4, 5} in which they injected parathormone into normal and pathologic human subjects and observed biochemical changes quite similar to those characteristic of patients with parathyroid tumors and by

* Read before the Association of American Physicians, Atlantic City, May 6, 1931.

their animal experiments⁶ in which they demonstrated by gross examination a loss of bone trabeculae as a result of chronic injections of parathormone.

Our aim was to provide a complete study of gross, roentgenologic and histologic changes in the bones of animals, subjected to chronic injections of parathormone, to determine whether the lesions were characteristic of one or more of the bone diseases mentioned and to observe whether the simultaneous administration of irradiated ergosterol would influence the results. Our interest in the effect

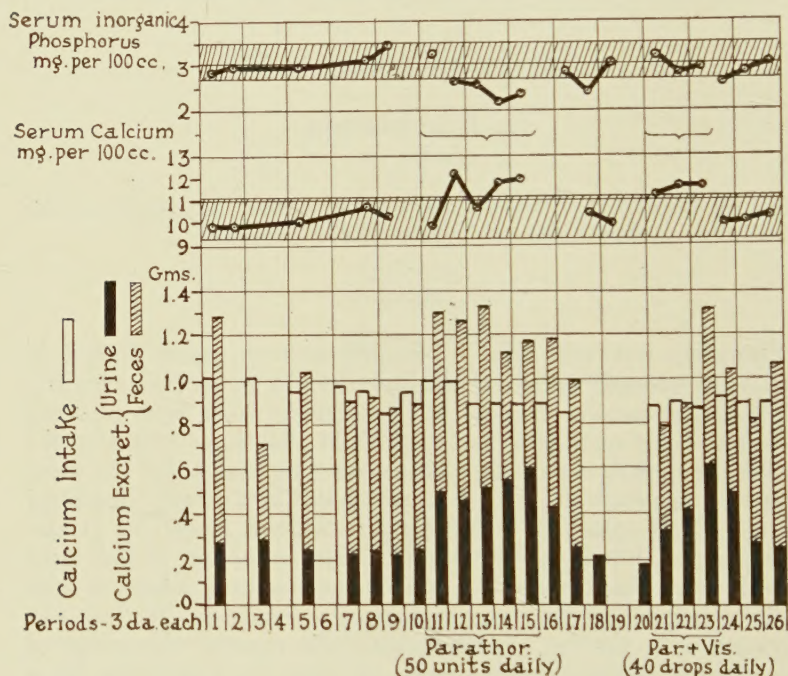


FIG. 1.—Parathormone experiment, October 14 to December 31, 1930, J. L. J., ♂, 35 years; height, 64½ inches; weight, 131 pounds.

of the ergosterol was aroused by incompletely controlled observations in a case of fibroid osteitis⁷ which had led us to infer that the course of this disease had been favorably influenced by vitamin D, also by the fact reported by Nonidez and Goodale,⁸ Higgins and Sheard,⁹ that deprivation of vitamin D led to parathyroid hyperplasia. There was a hint in this that fibroid osteitis was closely related to rickets and osteomalacia.

The study was made the subject of a thesis for the doctorate of philosophy by J. L. Johnson and the details of these experiments will be reported elsewhere by him. He began them in July, 1929, and had obtained very convincing results before we learned of the

similar studies of Jaffé and Bodansky. Johnson's¹⁰ observations completely confirm those of Jaffé and Bodansky.^{11,12} The lesion produced by chronic injections of Collip's parathormone prepared by the Eli Lilly Company is a lacunar resorption of bone which involves both the trabeculae and the corticalis, and is accompanied by the replacement of both marrow and bone by fibrous connective tissue, the process including cyst formation and the appearance of giant cells, and resulting in a histologic picture that so closely resembles the generalized osteitis described by von Recklinghausen²

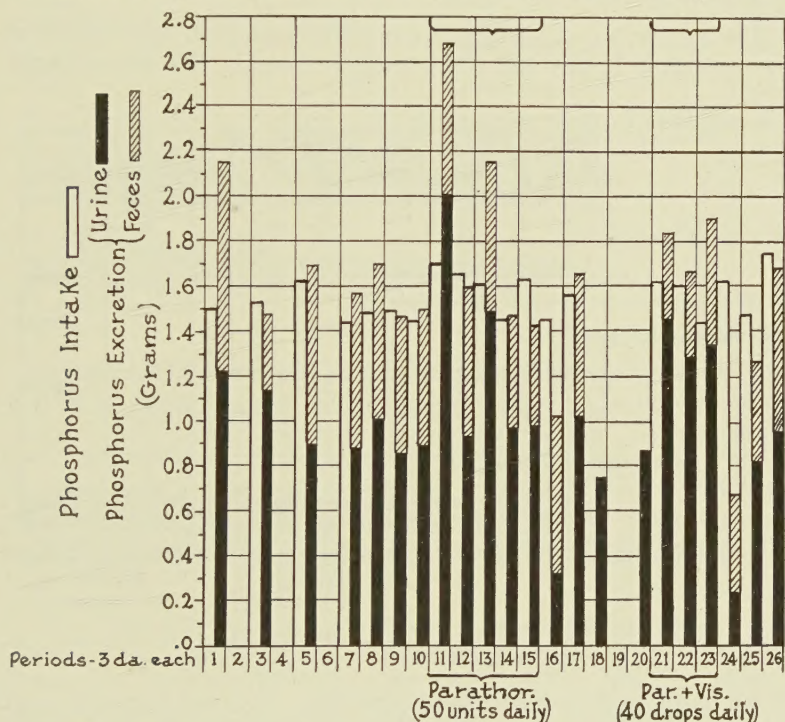


FIG. 2.—Parathormone experiment, October 14 to December 31, 1930, J. L. J., ♂, 35 years; height, 64½ inches; weight, 131 pounds.

as to be practically identical with it. No lesion has resulted, in over 100 experiments in puppies, young dogs and rats, which bore so close a resemblance to osteomalacia, to rickets, or to any other bone disease. The experiments further reveal that the effects of parathormone injections are not counteracted by the simultaneous administration of irradiated ergosterol.

The dosage of parathormone in the case of the rats was usually 20 units a day and when viosterol was added, its dose was usually 15 drops a day. In the case of the puppies the parathormone dosage was increased progressively from 2 to 20 units a day. The viosterol given to puppies was 5 to 20 drops daily. It will be recalled that the

rat has been considered to be unusually resistant to parathormone. Our experiments show that this resistance is only relative and that proper doses of the hormone are definitely active.¹⁰

Two calcium and phosphorus balance experiments were included as a part of this study. These were made on two human subjects with a view to observing the biochemical and symptomatic effects of daily injections of parathormone, and in order to discover whether these would be influenced by treatment with irradiated ergosterol. The results in one of these experiments we wish to report at the present time.

The subject was a healthy, male, colored student, aged 35 years, 64.5 inches in height and 131 pounds in weight. He was engaged

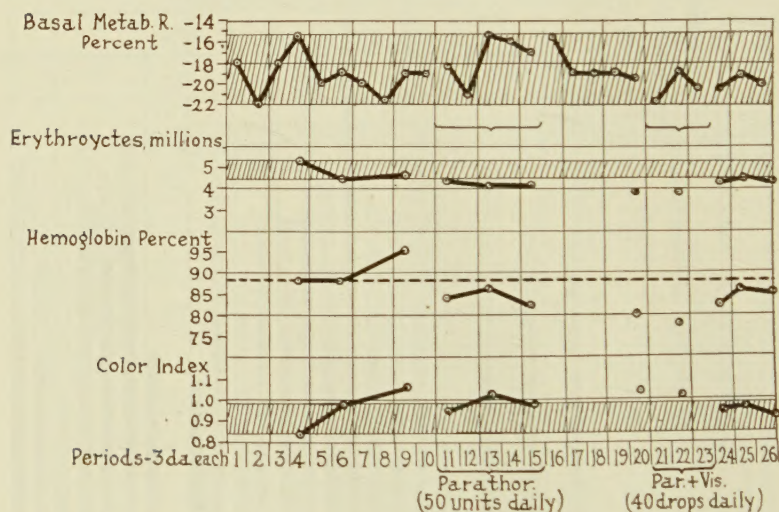


FIG. 3.—Parathormone experiment, October 14 to December 31, 1930, J. L. J., ♂, 35 years; height, 64½ inches; weight, 131 pounds.

in laboratory duties during the entire time. The technique followed in general that developed at the Massachusetts General Hospital,¹³ differing, however, in that Bauer and Aub's diets had been rigidly limited in calcium (to about 0.1 gm.) in order to minimize the significance of unmetabolized calcium, whereas our diet was planned to contain just sufficient calcium, about 0.4 gm., to maintain a calcium equilibrium. The urine throughout the experiment was neutral to litmus, indicating a reasonable balance of acid base values in the diet. A slightly positive nitrogen balance existed throughout the experiment.

The diet was held rigidly constant for 78 days and actual control of it was maintained by direct analysis of 10 per cent aliquot portions (Table 1). These analyses were made in 20 of the 26 three-day periods. The fluid intake was held constant at 2000 cc. and the sodium chlorid at 3 gm. All water used for drinking, for the prepa-

ration of food, and for washing containers for food or excreta was distilled.*

TABLE 1.—COMPOSITION OF THE DIET.

Parathormone Experiment, October 14 to December 31, 1930.

Subject: J. L. J., aged 35 years; height, 164 cm.; weight, 62.2 kg.; diet, October 21 to December 31.

Food.	Grams.	Food.	Grams.
Orange juice	100	Butter	55
Apple sauce	200	Salad oil	10
Lemon juice	60	Egg white	120
Sugar	135	Steak	40
Ginger ale	200	Cheese	15
Lettuce	60	Cream, 40 per cent	100
Celery	30	C. P. Salt	3
Bread	120	Total fluids	2000
		Nitrogen, gm.	Calcium, gm.
By calculation	2215*	6.73	0.396
By analysis of { Max.		7.69	0.336
10 per cent { Min.		6.69	0.286
aliquots† { Mean		7.04	0.307
* Basal metabolic rate	1230		0.522

† Aliquots of the food were prepared daily. These were combined in 3-day periods and analyzed. The diet of the entire experiment was controlled by such direct analysis.

The experiment consisted of a control or fore period of 31 days, a period of parathormone administration, in a daily dosage of 50 units, which lasted 15 days, a recovery period of 15 days, a period of 9 days with parathormone in the previous dosage plus 1 cc. of viosterol and a final recovery period of 9 days. Urine and feces were collected for analysis in 3-day lots, carmin alum markers serving for separation of the feces. The analytical methods are tabulated.^{13, 16, 17, 18, 19} (Table 2.)

TABLE 2.—ANALYTICAL METHODS FOR METABOLISM STUDIES.

	Phosphorus.	Calcium.	Nitrogen.	Creatinin.
Urine . . .	Fiske and Subbarow	Aub's modification of McCrudden	Kjeldahl	Folin
Feces . . .	Fiske and Subbarow	McCrudden	Kjeldahl	
Food . . .	Fiske and Subbarow to filtrate of ash	McCrudden	Kjeldahl	
Blood serum	Fiske and Subbarow	Clark and Collip modification of Kramer-Tisdall		

The biochemical results are shown in the accompanying charts and Table 3. The response to parathormone, like that previously reported by Collip,¹⁴ Greenwald and Gross¹⁵ and others, consisted of an elevation of the calcium and depression of the phosphorus of the serum, and a prompt, very markedly increased urinary

* The details of the preparation and serving of the diets used in this and other experiments to be reported from this laboratory, the preparation of aliquot portions of the diet, as well as the methods of collection and drying of excreta, will be the subjects of separate papers by Florence Smith, Lillian Eichelberger and their associates.

excretion of phosphorus and calcium. The data tend to support the view of Aub and his associates,⁴ and of Albright and Ellsworth,⁵ that the phosphorus metabolism is affected noticeably earlier than the calcium. The administration of viosterol with parathormone gave similar results except for a slight delay in the development of the maximal urinary excretions, both of phosphorus and of calcium. There was also some indication that a greater amount of both calcium and phosphorus were absorbed from the bowel. However, the effect of viosterol in this dosage was certainly not such as to demonstrate an antagonism in its action to that of the parathormone.

Daily determinations of the creatinin excretion, the nitrogen balance and the basal metabolic rate failed to indicate any metabolic alterations apart from those of the mineral metabolism. (Table 3.) A moderate anemia was observed, comparable to that reported in patients with parathyroid tumors. A very slight diuresis of short duration was observed as a result of parathormone administration.

The symptomatic response to medication with parathormone of this dosage (50 units daily) was of unusual interest. Muscular weakness appeared on the 12th day of the parathormone period. It was associated with a dull aching in, and tenderness of, muscles and bones of the arms and legs. Sharp, shooting pains followed, and a dull headache was complained of. These symptoms almost disappeared in the first recovery period but became worse than ever in the subsequent parathormone viosterol period, accompanied then by a sense of depression and loss of ability for mental work. Bone tenderness and muscle weakness were observed by Johnson¹⁰ in his experimental animals. They are characteristic symptoms of fibroid osteitis.

Osteitis fibrosa osteoplastica has thus been reproduced experimentally in animals, in almost every particular, by the injection of parathormone. This convinces us of the correctness of the conclusion that the pathogenesis of this disease, as it is observed spontaneously in man, is an oversupply of parathyroid hormone. Lesions other than those characteristic of fibrous osteitis did not occur in over 100 chronic experiments with animals, from which we infer that the hypertrophies and hyperplasias of the parathyroid glands, noted in skeletal diseases other than fibroid osteitis, are secondary phenomena. The fact that viosterol neither significantly alters the disturbed calcium and phosphorus metabolism of hyperparathyroidism, nor modifies the histological abnormality of the bones of animals receiving chronic injections of parathormone, is evidence that osteitis and osteomalacia (rickets) are of different origin.

Summary. The repeated injection of parathormone has produced, in puppies and young rats, a uniform skeletal abnormality that is characteristic of osteitis fibrosa osteoplastica (von Recklinghausen). A lacunar resorption of the trabeculae and corticalis of bones resulted, with replacement of marrow and cortex by fibrous connective tissue and osteoid tissue. Giant cells appeared and

TABLE 3.—METABOLIC DATA IN PARATHORMONE EXPERIMENT, OCT. 14 TO DEC. 31, 1930.
Subject: J. L. J. (Male), 35 yrs. Height, 164 cm. Average weight 59 kg.

Period (3 days each).	Calcium.				Phosphorus.				Nitrogen.				Urine.		Serum.				B.M.R., per cent.		
	Output.				Output.				Output.				Cre- ati- nin, gm.	Calcium.		Phosphorus.					
	In- take, total gm.	Bal- ance, gm.	In- take, total gm.	Bal- ance, gm.	In- take, total gm.	Bal- ance, gm.	In- take, total gm.	Bal- ance, gm.	In- take, total gm.	Bal- ance, gm.	In- take, total gm.	Bal- ance, gm.		Vol., cc.	Max., mg.	Min., mg.					
																	Urine, gm.	Feces, gm.		Total, gm.	Urine, gm.
I	1.018	0.274	1.021	1.295	1.503	1.227	0.936	2.163	-0.660	20.1	22.2	2.6	24.8	-4.7	8105	4.06	9.3	2.9	2.8	-18	
II	1.017	0.296	0.428	0.724	1.530	1.142	0.338	1.480	+0.050	20.2	17.4	1.4	18.8	+1.4	6625	3.83	9.9	2.9	2.9	-22	
III	0.950	0.248	0.801	1.139	1.637	0.901	0.792	1.693	-0.056	20.4	17.0	2.8	19.8	+0.7	6310	4.16	10.4	3.1	2.7	-16	
IV	0.978	0.233	0.792	1.025	1.446	0.880	0.697	1.577	-0.131	20.6	17.5	2.1	19.5	+1.0	5920	4.15	10.4	3.1	2.7	-20	
V	0.966	0.249	0.792	1.041	1.484	1.016	0.697	1.713	-0.229	20.7	17.4	2.1	19.5	+1.0	6260	4.01	10.4	3.4	2.7	-20	
VI	0.806	0.231	0.653	0.884	1.494	0.866	0.598	1.464	+0.030	20.1	16.8	2.1	18.7	+1.3	5610	4.11	10.5	3.5	3.4	-21	
VII	0.917	0.252	0.653	0.903	1.454	0.899	0.598	1.497	-0.043	20.6	16.8	2.1	18.9	+1.7	6165	3.99	10.2	3.5	3.4	-19	
Parathormone started; 25 units twice daily.																					
XI	1.009	0.500	0.800	1.300	1.717	2.012	0.674	2.686	-0.969	21.6	16.6	2.0	18.6	+3.0	6580	4.05	12.1	10.0	3.5	3.1	-20
XII	0.989	0.464	0.800	1.264	1.658	0.940	0.674	1.614	+0.044	20.2	16.1	2.0	18.0	+2.1	5040	3.97	12.4	11.5	2.7	2.4	-21
XIII	0.893	0.519	0.800	1.319	1.618	1.489	0.674	2.163	-0.345	21.0	17.2	2.0	19.2	+1.9	7180	4.01	11.0	10.7	2.6	2.2	-16
XIV	0.898	0.566	0.565	1.131	1.456	1.028	0.448	1.476	-0.020	21.0	16.7	1.4	18.1	+2.9	6380	3.72	11.8	10.9	2.6	2.1	-15
XV	0.885	0.610	0.565	1.175	1.639	0.987	0.448	1.435	-0.204	21.1	16.9	1.4	18.2	+2.9	5930	3.82	11.8	11.0	2.8	2.3	-17
Discontinued parathormone.																					
XVI	0.893	0.433	0.748	1.184	1.464	0.393	0.639	1.032	+0.432	20.7	16.0	2.0	17.9	+2.8	5320	4.95	10.5	10.5	2.9	2.9	-17
XVII	0.857	0.264	0.748	1.012	1.571	1.023	0.639	1.662	-0.091	20.9	17.0	2.0	19.0	+1.9	6145	3.92	10.5	10.5	2.4	2.4	-19
XVIII	0.875	0.235	0.699	0.936	1.447	0.755	0.558	1.313	-0.091	20.9	17.0	2.0	19.0	+1.9	6190	3.68	10.5	10.5	2.4	2.4	-19
Violesterol started; 40 drops daily.																					
XIX	0.875	0.235	0.699	0.936	1.447	0.755	0.558	1.313	-0.091	20.9	17.0	2.0	19.0	+1.9	6190	3.68	10.5	10.0	13.0	3.0	-19
XX	0.188	0.188	0.188	0.188	0.874	0.874	0.874	0.874	0.874	16.7	16.7	16.7	16.7	16.7	6465	4.20	10.0	13.0	3.0	3.0	-19

* Blood drawn 2 hours after breakfast.

† Blood drawn 6 hours after initial injection.

‡ Blood drawn before first parathormone injection.

§ Only 1 determination during period.

TABLE 1.—DIAGNOSTIC FEATURES OF HYPERPARATHYROIDISM AND OSTEITIS FIBROSA OSTEOPLASTICA.

I. More frequent findings:	II. Less frequent findings:
1. Increased urinary excretion of calcium.	1. Bone deformities.
2. Negative calcium balance.	2. Multiple cysts.
3. Hypercalcemia.	3. Pathologic fractures.
4. Hypophosphatemia.	4. Giant cell tumors of bone.
5. Parathyroid hypertrophy or hyperplasia.	5. Impaired kidney function.
6. Rarefaction of bones.	
7. Decreased muscular response to stimulation.	
8. Muscular weakness and hypotonia.	
9. Muscle and bone pains.	
10. Anemia.	

Waltner³⁴ attempted to prove that hyperfunction of the parathyroid glands was responsible for rickets. He experimented with young white rats, using three diets. In one of these the calcium and phosphorus contents were normal, in one phosphorus was deficient, a decidedly rachitic diet and in one calcium was deficient, a somewhat rachitic diet. Each rat received 20 units a day of Collip's parathormone for from 8 to 18 days. With the normal diet an osteoporosis was evident, but no rickets. With the deficient diet both rickets and osteoporosis developed. In no case did the controls receiving the normal diet develop rickets, but some of the controls on the deficient diet did. Waltner concluded that while parathormone would wash calcium from the bones of young rats it did not produce rickets.

Bauer, Aub and Albright⁵ gave parathormone to rabbits, cats and rats and studied its effects by gross examination and with roentgenograms. A study of the blood serum calcium was included. Their rabbits revealed a reduction in the number of trabeculae but no gross changes of the cortex of bones. Histologic examinations were not made. In growing kittens no greater reduction of trabeculae was observed than occurred in the course of normal bone growth. The skeletons of the rats appeared, by roentgenogram, to be much denser and shorter than those of their control siblings, and the number of trabeculae was found to be increased on gross examination of the sectioned bones. A rise in serum calcium was not observed in cats or kittens, but a slight rise was secured with rats and rabbits.

Jaffe, Bodansky and Blair^{22,23} have recently reported a very complete study of the effect of repeated injections of parathormone in dogs and guinea pigs. The histologic examination of the skeletons of their animals revealed lesions that are characteristic of osteitis fibrosa. In most cases the serum calcium was increased and the serum phosphorus decreased by the parathormone injections, but skeletal lesions occurred without hypercalcemia or hypophosphatemia.

Numerous other reports of parathormone injections in animals and man are contained in the literature, but as far as has been

ascertained those referred to are the only ones in which attention has been directed to lesions of the bones.

My preliminary experiments,²⁴ which resulted in the production in rats and in puppies of bone lesions characteristic of osteitis fibrosa were completed before the work of Jaffe, Bodansky and Blair was brought to my attention. A chronic state of hyperparathyroidism was produced in rats and dogs by repeated injections of parathormone and the skeletal abnormalities were studied grossly, roentgenologically and microscopically. The effect of this procedure on serum calcium and phosphorus and metabolic balances of calcium, phosphorus and nitrogen were studied in separate experiments conducted on man, as previously communicated.²⁶ The data obtained with dogs will be reported later. The observations with rats is the subject of the present paper.

Methods. White rats from 6 to 12 weeks old were used, young animals being chosen for the reason that growing bones would probably reveal more striking abnormalities. Steenboeck's normal rat diet was given. This consisted of yellow cornmeal, 76; linseed meal, 16; crude casein, 5; ground alfalfa, 2.5; sodium chlorid, 0.5 and calcium carbonate, 0.5 per cent. Distilled water was supplied for drinking. The food and water were kept in the cage at all times. This diet was started 1 or 2 weeks before treatment began. A number of animals received varying amounts of parathormone in the effort to arrive at a satisfactory dosage, the dose finally adopted being one of from 10 to 20 units injected subcutaneously. Animals were started with 10 units, this was increased after about 5 days to 15 units, and after a week or 10 days to 20 units. Twenty days was about the average duration of an experiment, although some rats received parathormone for as long as 43 days. Control litter mates were maintained on the same diet and under the same conditions as injected animals.

In certain cases when definite skeletal abnormality had become visible grossly and by roentgenograms, the medication was discontinued to observe the recovery. The diet in these cases was not altered.

Roentgenograms of the entire skeleton of all animals were made at death or at killing. In several instances periodic roentgenograms were obtained during the course of the experiment.

The soft tissues were examined grossly and some of these were prepared for microscopic study. All of the long bones were dissected out, fixed in formalin and decalcified with 5 per cent nitric acid. Tissues for microscopic examination were embedded in paraffin and stained with hematoxylin and eosin. Mallory's connective tissue and van Gieson's stains were also used.

Results. A total of 32 rats were given parathormone in the course of these experiments with very uniform results. The protocols of several representative animals are given in tabular form to illustrate the variations observed. (Table 2.)

The most outstanding gross abnormalities were bowing of the forelimbs and muscular weakness. The time of the first detectable evidence of bowing varied from 6 to 20 days, with 12 and 15 days as an average for the production of definite deformity. The extent of this is illustrated. (Fig. 1.) A loss of muscle tone was apparent on handling the animals. Muscular weakness was also revealed by their slow limping movements and their inability to climb up the sides of cages.

TABLE 2.—SOME RESULTS OF PARATHORMONE ADMINISTRATION TO RATS.

Rat No.	Sex.	Days on experiment.	Parathormone administration.		Gross observations.†	Roentgenological observations.†	Microscopic observations.‡
			Dose,* in units.	Total units.			
1 . .	M	42	10 ³² (⁴²)	330	Toes on left forefoot drawn like clenched fist; ³ does not bear weight on left foot; growing well; condition remained good throughout; killed ⁴²	++ Long bones moth-eaten appearance at epiphyses; suggestion of kidney outline	++++ Decalcification, resorption of cortical and trabecular bone, fibrous replacement, bone and marrow, cyst formation, giant cells, osteoid tissue; one area of highly vascular fibrous tissue.
2 . .	M	42	10 ⁷ (⁸) 20 ⁸ 15 ²² (²⁶)	475	Agglutination of eyelids, red material around eyes like dried blood; ¹¹ eyes cleared, ¹² condition good; ¹² beginning bowing and knobbing; ¹² killed ⁴²	+ Rarefaction of tail vertebrae; slight rarefaction of long bones	++ Decalcification, bone resorption, bone and marrow fibrous, cysts, giant cells, osteous tissue.
3 . .	F	42	10 ⁷ (⁸) 20 ⁴ 15 ²² (²⁷)	480	Condition good; no appreciable change at end of experiment; killed ⁴²	++ Rarefaction of long bones; curvature of left tibia	+ Decalcification, bone resorption, fibrosis of bone and marrow, cysts, numerous marrow giant cells.
12 . .	M	28	10 ⁴ (²⁶) 15 ⁷ (⁸)	245	Agglutination, red material around eyes; ⁶ muscular weakness and hypotonia, ⁶ slightly bowing of forelimbs and knobbing at wrists ⁶	++ Moth-eaten appearance at epiphyses; pathologic fracture; rarefaction of medullary and cortical bone; slipped epiphysis	++ Decalcification; bone resorption; fibrosis of bone and marrow, cysts, osteous tissue.
B . .	F	7	40 ⁴ (⁷)	160	Bowing of forelimbs and knobbing of wrists; ⁶ muscular weakness and hypotonia, ⁶ found dead ¹	+ Moth-eaten appearance at epiphyses	+++ Decalcification, necrotic areas in marrow; circumscribed area of fibrous tissue and giant cells in medullary cavity, cysts, fibrosis of bone and marrow; in region of epiphyseal cartilage, large area of fibrous tissue and

22 . .	F	12	15 ⁽¹⁰⁾	90	Firm agglutination of both eyes; ¹¹ muscular weakness and hypotonia; ¹¹ found dead and almost completely eaten by litter mate. ¹²	+ Moth-eaten appearance of epiphyses; slipped epiphyses	No sections.
23 . .	F	276	15 ¹⁴⁽¹⁹⁾ 20 ¹⁵⁽¹⁶⁾	515	Nasal hemorrhage; ³ suggestive knobbing of wrists and bowing of forelimbs; ¹⁶ muscular weakness and hypotonia; ¹⁹ definite knobbing and bowing; ²³ bowing very great, otherwise condition good; ³⁶ treatment discontinued to note changes in deformities on diet alone; ⁴⁰ deformities gradually disappeared and finally could not be detected except for a very slight knobbing of right wrist; condition of animal was excellent up to end of experiment; killed ²⁷⁶	+ + + + Rarefaction; series of films show progressive destruction of bone and final film shows marked healing with persistence of slight deformity of right wrist, left radius and ulna and right tibia and fibula; suggestion of kidney outline	- Except for evidence of healed fractures and overgrowth of bone, histologic appearance was normal.
28 . .	F	15	15 ¹¹⁽¹²⁾	165	Bowing of forelimbs; ¹¹ agglutination of both eyes; ¹³ muscular weakness and hypotonia; ¹³ found dead ¹⁵	+ + + + Rarefaction; moth-eaten appearance of epiphyses; bone destruction; suggestion of kidney outline	No sections.

Control animals grew normally, remained in excellent condition and showed normal bones roentgenologically and histologically.

* The numerals used in this column as exponents and without parenthesis represent the number of injections of the specified dose, and the numbers within parentheses represent the number of days over which these injections were spread.

† The numerals in this column represent the day of the experimental period when the observation was made.

‡ One to four plus signs are used to give a relative idea of the extent of the lesion.

Agglutination of the eyes was seen not infrequently. The agglutinating material resembled dried blood. Blood-tinged and soft feces were noted occasionally and in 1 animal, Rat 23 (Table 2), nasal hemorrhage occurred after the third day.



FIG. 1.—Rat 21. Bowing of legs typical for rats receiving parathormone. Animal received 15 units of parathormone daily for 14 days and 15 drops of viosterol daily for 17 days. This photograph made on 19th day of experiment.

Roentgenologic examinations revealed rarefaction in the bones of all rats that had received parathormone for a period longer than 3 days. This gave a mottled appearance in most cases and involved first the ends of the long bones, later the shafts. (Figs. 2, 3, 4). The responsibility for these changes was more definitely fixed upon parathormone, and the diet was ruled out as a factor, by keeping all conditions the same after the changes appeared and discontinuing the injections. Rat 23 is typical of what then happened. The bowing disappeared and the only gross evidence of deformity was a knobbing of the right wrist which could be detected only by careful examination. The roentgenograms then showed no rarefaction or bone destruction (Fig. 5), but still some bowing of the left radius and ulna and of the right tibia and fibula, and an unevenness throughout their length of the right radius and ulna. The restitution appeared otherwise to be complete. I was able occasionally to detect a slipped epiphysis in the roentgenograms. In 1 animal there was also a slight scoliosis.

The roentgenograms of these animals do not reveal any definite intensification of shadows of the kidneys, although there is a suggestion of kidney outline in some cases. This feature will receive further comment in a subsequent paper upon the effects of the simultaneous administration of parathormone and irradiated ergosterol.



FIG. 2

FIG. 3

FIG. 2.—*Rat 23*. Bone destruction, especially at upper end of each tibia; pathologic fracture and cyst of head of humerus. Roentgenogram made on 26th day of experiment. Animal received 15 units of parathormone for 16 days and 20 units a day for last 4 days.

FIG. 3.—*Rat 23*. Roentgenogram (7 days after that of Fig. 2) shows destructive process further advanced as a result of continued treatment. Dosage of parathormone 20 units daily.



FIG. 4

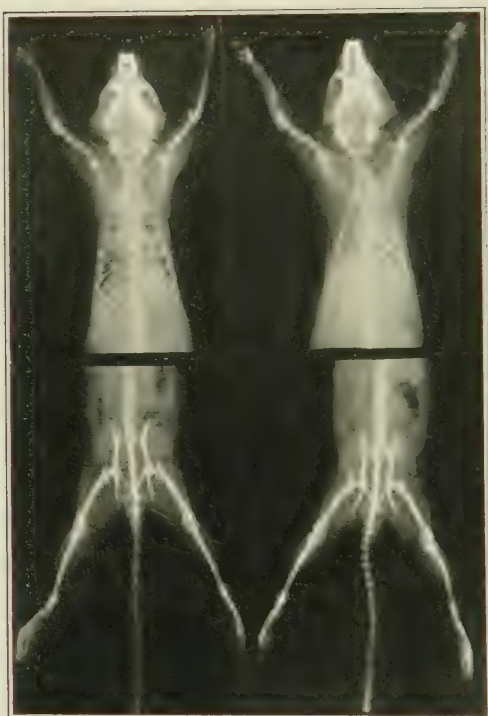


FIG. 5

FIG. 4.—*Rat 23*. Destructive process 14 days later than that of Fig. 2. Treatment was discontinued after this roentgenogram was made, animal at this time had same gross appearance as shown in Fig. 1. Dosage of parathormone 20 units daily.

FIG. 5.—*Rat 23*. Upper and lower limbs of animal by roentgenogram 8 months after treatment was discontinued, diet and other conditions remaining the same. Grossly the bowing had entirely disappeared. Except for slight curvature of some bones and exostoses of one foreleg, appearance of *Rat 23* by roentgenogram at this stage was no different from that of its control litter mate as shown on same film.

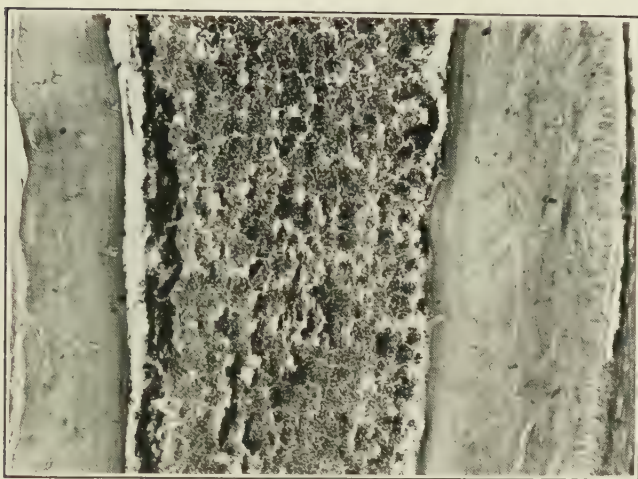


FIG. 6. *Rat S.* Appearance of bone and marrow in shaft of a control animal. ($\times 60$)

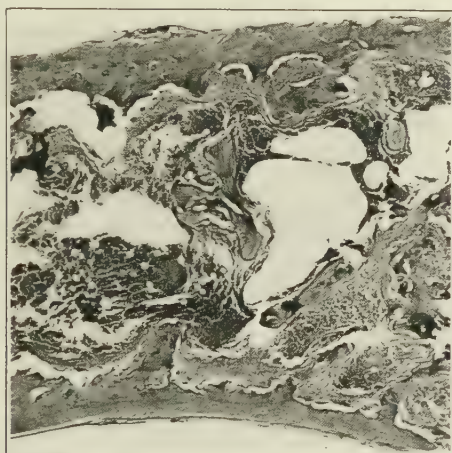


FIG. 7.—*Rat B.* Animal died on 7th day of experiment from overdosage of parathormone. Forty units of parathormone daily for 4 of the 7 days on experiment. Resorption of cortical bone and marrow fibrosis. Small cysts can be seen in the lymphoid marrow. ($\times 30$)

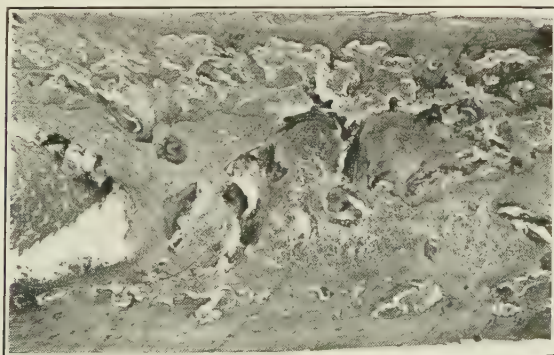


FIG. 8.—*Rat A.* (Parathormone and viosterol.) Lacunar resorption of cortical bone and marrow fibrosis. ($\times 26$) Typical picture for rats receiving parathormone. Animal died on 10th day of experiment; received 20 units of parathormone daily for 8 days and 15 drops of viosterol daily for same period.

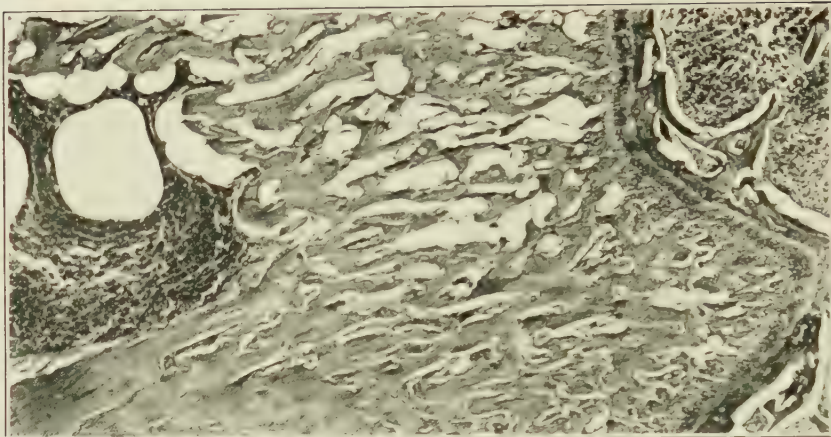


FIG. 9.—*Rat 2*. Animal killed on 42d day of experiment. Ten units of parathormone daily for 7 days, 20 units for 3 days and 15 units for 23 days. Normal appearing epiphyseal cartilage, proliferation of osteoid tissue, filling in of spaces by fibrous connective tissue and cysts in lymphoid marrow. ($\times 55$)

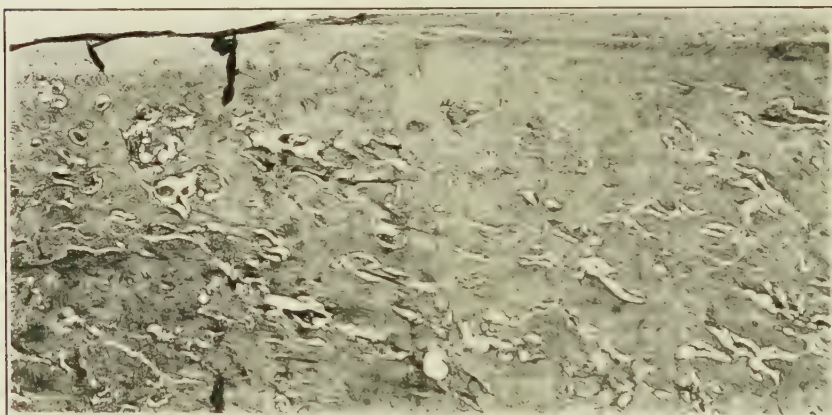


FIG. 10.—*Rat 1*. Animal killed on 42d day of experiment. Ten units of parathormone daily for 33 days. Abundance of osteoid tissue in shaft of long bone with numerous spaces filled by fibrous connective tissue. ($\times 30$)

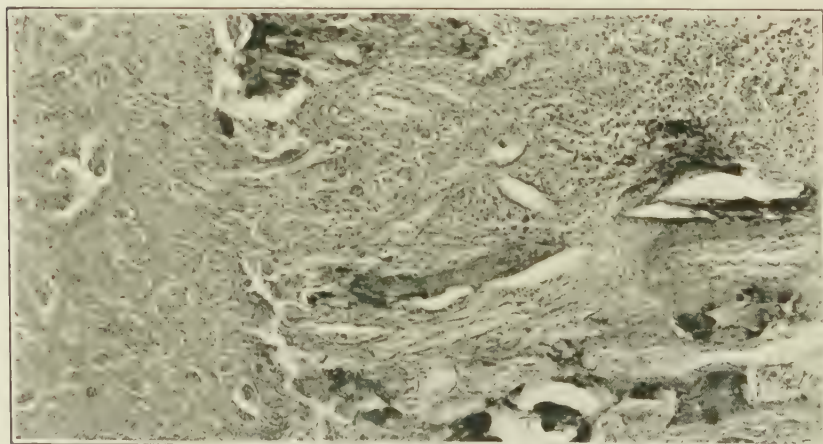


FIG. 11.—*Rat B*. Fibrous tissue, fibrous marrow and some osteoid tissue in region of epiphyseal cartilage. ($\times 140$) (Fig. 7, *Rat B*)

Histologically the long bones of the extremities of the parathormone-treated animals show decalcification, bone resorption, replacement of bone and marrow by fibrous connective tissue and cyst formation. These are constant findings. Giant cells resembling osteoclasts are present in large numbers and in close association with fibrous connective tissue. Giant cells are inconstantly observed in circumscribed areas of fibrous connective tissue, in the marrow cavity as well as in close proximity to bone. In some instances they appear in large number in masses of fibrous connective tissue suggesting the picture of the so-called giant cell tumor. Osteoid tissue is quite abundant in many of these skeletons and with the fibrous connective tissue almost obliterates the medullary cavity. Pathologic fractures are also numerous. The types of change observed are illustrated. (Figs. 6 to 11.) The microscopic studies of the soft tissues have not been completed, but those of the kidneys reveal metastatic calcium in the tubules and to a less extent in the interstitial tissue; not in the glomeruli.

Discussion. It was Collip's opinion, in 1926, that the rat was immune to parathyroid extracts, and this opinion has prevailed until recently. That this is not the case is evident both from my experiments and the experiments of Waltner (from which he concluded that parathormone injections wash calcium out of the bones but do not produce rickets), the experiments of Bauer, Aub and Albright (showing some bone changes after parathormone administration) and those of Rose and Stucky³⁰ (in which successive injections of parathormone produced significant increases in blood calcium).

Under the conditions of my experiments there invariably resulted osteoporosis, bone resorption, replacement of bone and marrow by fibrous connective tissue and cyst formation. New bone (osteoid tissue) occurred with great frequency. All of this is characteristic histologically of osteitis fibrosa osteoplastica, as observed clinically. The roentgenograms and gross findings of the animal experiments are equally those of osteitis fibrosa. These results with the rat correspond to those with puppies to be reported later and to the lesions produced in guinea pigs and in dogs by Jaffe, Bodansky and Blair.^{22, 23}

The bones of the rats treated with parathormone by Bauer, Aub and Albright were denser and shorter in roentgenograms, and when they were split and examined grossly appeared to contain an increased number of trabeculae. These observations are not in harmony with those I have made, but an explanation of the discrepancies is to be found, perhaps, in a difference in diet and a different dosage of parathormone. Bauer and his associates gave more calcium than my rats received, whereas their dose of parathormone was only about one-half as great as the dose I used. Under such circumstances new bone formation may keep pace with or exceed bone destruction and the newly formed osteoid tissue, which appears abundantly in many of my sections (Figs. 9 and 10), may

become more or less calcified. An increase in bone density was observable in some of my experiments on the dog.²⁵

Summary. White rats, aged from 6 to 12 weeks, were injected daily for periods of from 10 to 43 days with parathormone in doses of from 10 to 20 units. A Steenbock normal rat diet was given. Uninjected litter mates as controls remained well, whereas the injected animals developed, without exception, muscular weakness, hypotonia and skeletal lesions characteristic of osteitis fibrosa osteoplastica (von Recklinghausen), namely, a lacunar resorption of bone with softening and deformity, bending and multiple fracture. The cortex and marrow of these bones were largely replaced by fibrous connective tissue containing numerous giant cells, and new bone osteoid tissue was also in evidence in numerous cases.

Conclusion. Chronic hyperparathyroidism produced in rats by repeated injections of parathormone leads to bone changes which justify a diagnosis of osteitis fibrosa. These experiments support the conclusion that the cause of clinical osteitis fibrosa osteoplastica (von Recklinghausen) is an excess of the parathyroid hormone.

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EXPERIMENTAL CHRONIC HYPERPARATHYROIDISM.

III. OSTEITIS FIBROSA PRODUCED IN PUPPIES.

BY JOSEPH L. JOHNSON, M.D.,*

CHICAGO, ILL.

(From the Department of Medicine, University of Chicago.)

THE production of a chronic state of hyperparathyroidism in experiments on man and rats has been reported and the more important literature bearing on spontaneous and experimental hyperparathyroidism is reviewed in the previous papers of this series. The present communication deals with the effects of repeated injections of parathormone in the dog.

Methods. Puppies were used because of the probability that young bones would show more striking changes from a decalcifying procedure. Their average age was about 8 weeks. Mineral balance studies were contemplated† and for this reason a synthetic diet was given to the first group of these animals. It was composed of casein, 37.8; sucrose, 34.9; lard, 17.0; butter fat, 7.0; bone ash, 2.3 and a salt mixture, 1.2 gm. The salt mixture consisted of sodium chlorid, 10; calcium lactate, 4; magnesium, 4; ferric citrate, 1.0 gm.; iodine as potassium iodid, a few drops and vitamin B in Harris' yeast vitamin powder. This diet is Cowgill's² improvement upon Karr's diet for metabolism experiments with dogs. The animals treated with parathormone did so poorly on this regimen that it was soon discontinued. Swift's Silver Fur Kennel ration was then used for a short time and finally a supplemented meat diet consisting of ground lean meat, bread, orange juice and cod-liver oil.

With few exceptions all animals were photographed before the experiment started and at intervals during the experiment. Roentgenograms were made before and at intervals during the experiments. Roentgenograms of the dissected bones were obtained after the death or killing of the animals. The soft parts were examined grossly and some of them kept for future microscopic study. The long bones were split longitudinally and examined grossly. The split bone was fixed in formalin, decalcified in 5 per cent nitric

* The expenses of this research were met from the John D. Hertz Fund.

† It proved to be impractical to conduct metabolic observations on these small animals. The repeated collection of blood was difficult to make and of questionable influence on the course of the disease; quantitative collection of urine and feces was impossible. For this reason this phase of the problem was studied with human subjects, as has been previously reported.

TABLE 1.

Dog No. and sex	Days after operation	Parathyroid extract dose in units	Gross observations †	Röntgenologic observations	Microscopic observations
29 (Male)	62	51 ¹ 101 ¹ 153 ¹ 203 ¹	Bowing of forelimbs; shoulders turn outward; hind legs turn outward at hip; waddling gait; muscular weakness; ³⁸ muscular weakness increased; sits down to eat, drink and play; ³⁹ watery blood-tinged stools; ⁴⁰ in jump of 18 inches from cage to floor foreleg was injured so that animal could not place weight upon his forefoot for about 3 days; moving pictures made to show gait; ⁴¹ skin eruption; ⁴² animal quite playful and gained weight through experiment; killed; ⁴³ autopsy: fracture upper right humerus; purplish-red color each end both humerus and tibia, fibula very flexible, split humerus shows circumscribed brownish area corresponding to area of decreased density on Roentgen ray film; cortex thick and dense; cystic cavity at upper end right ulna 20 mm. to 3 mm; both ulnae curved.	Right upper ulna: 3 spots of decreased density 3 mm. wide; uppermost spot 10 mm. long; a third 6 mm. long; outline is quite definite and there are undoubtedly cysts; ulnae curved; left humerus: rarefaction; no evidence of cortex in region of shaft, distorted trabeculation, pathologic fracture; right humerus: distorted trabeculation at upper end, circumscribed area with finely mottled appearance; pathologic fracture, rarefaction and moth-eaten appearance of radii; femur shows increased and distorted trabeculation and cysts; fibula irregular and uneven; rarefaction of pelvis.	Upper ulna: fibrous tissue fills enlarged cortical canals, and some appears to be undergoing ossification; bone resorption; bone and marrow fibrosa, cysts, hemorrhagic into marrow spaces.
32 (Female)	..	51 ¹ 101 ¹ (11) 153 ¹ (11) 203 ¹	Muscular weakness, slight knobbling of wrists; ⁴⁴ very weak but quite playful; ⁴⁵ clouding of one cornea giving eye slate-blue appearance, cleared up after 1 week; animal stronger and more active at time of killing; ⁴⁴ condition of animal was good throughout experiment; autopsy revealed nothing of significance.	Very slight rarefaction, multiple small faintly visible cysts, especially numerous in pelvis; increased trabeculation in long bones, thinning of cortex, irregular inner margin of cortex, healed pathologic fractures	Pathologic fractures; necrotic areas in bone marrow; numerous large marrow cells, not same as giant cells, resembling osteoclasts; vascularized callus; mild bone and marrow fibrosis; numerous giant cells.
51 (Male)	9	24 51	Shows signs of falling; ⁴⁶ very weak; ⁴⁷ found dead; ⁴⁸ autopsy showed death most likely due to pneumonia.	Suggestion of cysts in metatarsal and phalangeal bones, otherwise dissected bones appear normal by Roentgen ray.	Slight bone resorption; dense vascular marrow; tendency to marrow-fibrosis; hemorrhage into marrow spaces; enlargement of cortical canals; some vessels in cortex show definite thrombosis; a cystic space in one of the long bones is filled with fibrin and

33 (Male)	59	21 57 15 ³ 20 ¹⁰	575	575
			Bowing of front legs; left hind leg turns outward at hip; genu valgum,⁴³ striking muscular weakness, especially in hind legs; deformity of left foreleg;⁴⁴ moving picture made;⁴⁷ tenderness in region of left shoulder; yelps when lifted by placing hands under arms;⁴⁸ found dead;⁴⁹ autopsy: split humerus shows brownish area 50 mm. x 6 mm. which corresponds in size and position to area of decreased density on Roentgen ray film; marrow hemorrhage discernible; numerous brown circumscribed areas in several bones which correspond to areas of decreased density on Roentgen ray films these are undoubtedly cysts; cortex appears quite thin and deep red color; most likely red marrow and blood shows through cortex; pathologic fracture of ulna; lungs edematous and abundant frothy material in trachea; death probably due to pneumonia; fibula flexible.	
45 (Female)	65	5 ¹¹ 10 ¹¹ 15 ¹⁵ 20 ¹⁹	770	
			Watery, blood-tinged stools;²⁰ tremors of entire body;²² marked curvature of left leg with convexity on medial aspect; appetite poor; marked muscular weakness and hypotonia; treatment suspended for 3 days;⁴⁵ treatment resumed, cats well, still very weak; cannot stand up to eat or to defecate; plays with other animals by crawling on abdomen;⁴⁸ stronger and now walks a little;⁴⁶ unable to stand; moving picture made;⁴⁷ killed;⁴⁶ autopsy: widened epiphyseal cartilage; marrow hemorrhage; small dark brown circumscribed areas in end of ulnae and radii and in left femur; bones bleed at ends when pressed gently with fingers; fibulae and pelvis very flexible.	Fibula curved and irregular widened epiphysis; rarefaction; left ulna shorter and wider than right; pathologic fracture; thinned cortex; epiphyseal separation; bowing; left radius shorter and narrower than right; ends of bones have moth-eaten and mottled appearance; iliac crest irregular and rarefied; cyst in ilium; fracture of pelvis anterior to acetabulum Bone resorption; fibrosis of bone and marrow; giant cells; marrow hemorrhage; overgrowth of cartilage; isolated patch of cartilage undergoing fibrosis.

* The numerals in this column used as exponents and without parentheses represent the number of injections of the specified dose; and the numerals within parentheses represent the number of days over which these injections were spread.

† The numerals in this column represent the day of the experimental period when the observation was made.

acid, mounted in paraffin and stained with hematoxylin and eosin. In some instances Mallory's connective tissue and van Gieson's stains were used.

Parathormone was administered subcutaneously in doses ranging from 2 to 20 units daily. In order to keep the animals alive for long periods it was found to be necessary to begin with small doses and increase gradually. The average duration of the experiments was about 35 days, although some animals lived for only a few days and some of the experiments were continued for several months. Many animals were lost from over-dosage and other causes before correct conditions were established. A chronic state of hyperparathyroidism which was long enough in duration for bone pathology to develop was the objective. The diet, the dosage of parathormone and the environment all proved to be important factors for success. The supplemented meat diet proved best. Warm, dry, clean and well ventilated quarters were found to be indispensable. The dosage of parathormone would depend upon the age of the animal and its condition. The younger animals tolerated large doses badly. In every case very small doses were used at first and the amount gradually increased. A control animal of the same age, usually a litter mate, was given the same diet and maintained in the same cage with each experimental animal.

Results. A total of 35 puppies were given parathormone in the course of these experiments. Abstracts of the protocols of a few experiments selected to cover the range of observations are given in tabular form. (Table 1.) The details of earlier studies were reported last year as a dissertation for the doctorate in medicine.

It was of special interest that 5 puppies that had been treated with parathormone for varying periods developed tonic and clonic spasms when the drug was discontinued. This tetany had the earmarks of parathyroid insufficiency. It caused the death of all 5. The serum in 1 of these was found to contain 10 mg. of calcium for each 100 cc. It was not examined in the other animals.

The abnormalities noted in all of these experiments were strikingly similar. Muscular weakness, hypotonia and bone deformities were invariable. Action pictures demonstrate muscular weakness by the limping gait, inability to jump and climb and frequency of resting. Gross observations revealed lateral bowing of the forelimbs and knobbing of the wrists of the majority of these animals (Figs. 1, 2 and 3). The roentgenograms show rarefaction, moth-eaten appearing areas, increased and distorted trabeculation and cysts of the long bones (Figs. 4 and 5). In some cases the pelvis was likewise involved. In some instances cysts were large enough to be plainly visible to the unaided eye when the long bones were split (Fig. 6). The most outstanding histologic changes were osteoporosis, resorption of both cortical and trabecular bone, enlargement of Haversian and communicating canals, the filling of these spaces by fibrous connective tissue, proliferation of osteoid tissue and cyst formation. Giant cells with numerous small, round nuclei appeared in large numbers, associated with fibrous connective tissue as well as in close proximity to bone which was undergoing resorption (Fig. 8).



FIG. 1.—Dog 6. Bowing of forelimbs after parathormone administration. Animal received a total of 46 units of parathormone in doses of from 2 to 8 units a day, and during first 7 days of experiment a total of 24 drops of viosterol in doses of from 3 to 5 drops a day. Medication was entirely discontinued on the 15th day of experiment and this photograph taken on the 16th day.



FIG. 2.—Dog 2. Knobbing of wrists following parathormone administration. Sixteenth day of experiment. Animal started on 12 units daily and reduced on 3d day to 8 units daily. Injections were discontinued on 9th day because of failing condition of animal.

Only in Dog 45 was there any apparent disturbance in growth of the epiphyseal cartilage. In this animal a marked overgrowth of cartilage cells suggested rickets, but even here there was a marked associated replacement of bone and marrow by fibrous connective tissue, as well as bone resorption and proliferation of osteoid tissue.



FIG. 3.—Dog 45. Deformity of forelimbs following parathormone administration. Photographed on 41st day of experiment. Started on 5 units daily and gradually increased to 20 units.

An occasional but infrequent observation was a slate-blue coloration which completely obliterated the transparent appearance of the cornea.

Discussion. With suitably nourished and well-cared-for puppies and proper precautions regarding dosage, it is thus possible to reproduce experimentally, by repeated injections of parathormone, a clinical picture that closely resembles the osteitis fibrosa osteoplastica described in man by von Recklinghausen. Similar results have been obtained with the rat⁴ and by Bodansky and Jaffe⁵ in the guinea pig and dog. A wide variety of symptoms and abnormalities had been noted in the human disease. In the experiments described on rats, dogs and man the repeated injection of parathormone has caused practically every peculiarity that has been noted clinically. Not all of these changes have been observed in any one animal any more than all have been observed clinically in any



FIG. 4.—Dog 6. Cysts in humeri.

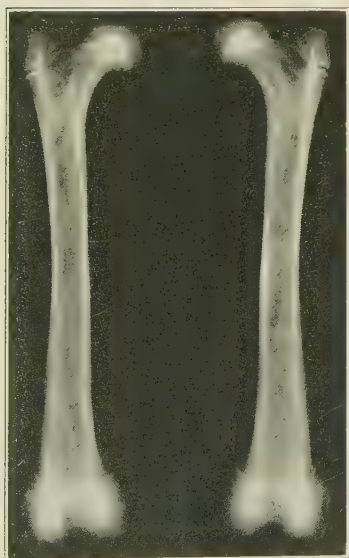


FIG. 5.

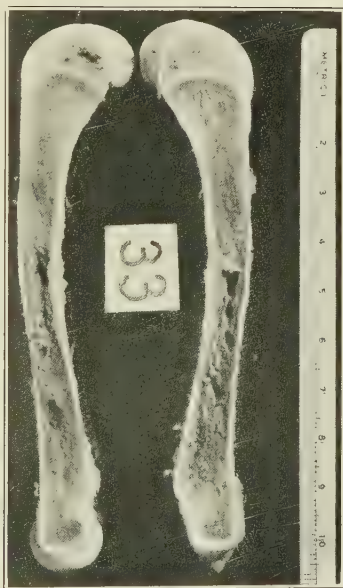


FIG. 6.

FIG. 5.—Dog 29. Left and right femur. Multiple cysts. Illustrations in Fig. 5 of Dog 29 were made at the termination of the experiment on the 62d day. Animal had received from 5 to 20 units of parathormone daily for a total of 720 units.

FIG. 6.—Dog 33. Two halves of right femur showing gross appearance of cysts. Five to 20 units of parathormone daily for a total of 575 units. Animal died on 59th day of experiment, at which time photographs were made.

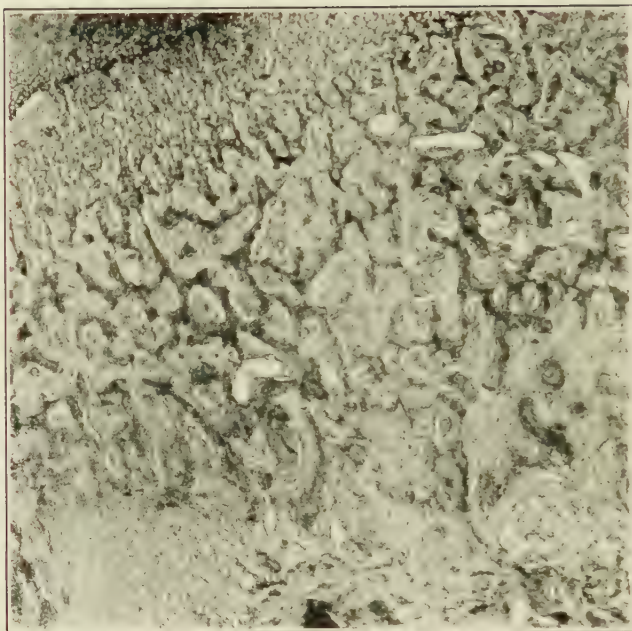


FIG. 7.—Dog 45. Animal killed on 65th day of experiment. Five to 20 units of parathormone daily, total 770 units. Lower end of femur. Principally fibrosis and osteoid tissue. ($\times 30$.)

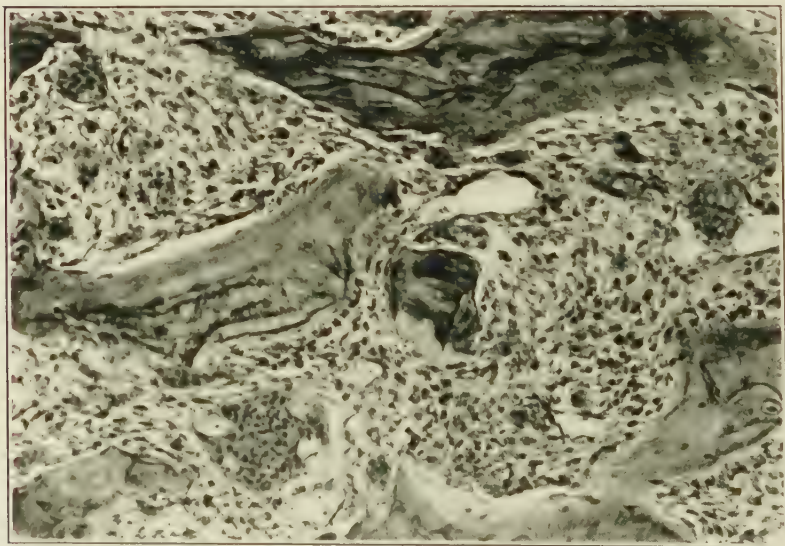


FIG. 8.—Dog 42. Animal received 5 to 20 units of parathormone daily, total 777 units; and 40, later 20, drops of viosterol daily, total 1340 drops. Killed on 67th day of experiment for histologic study of bones. Three multinucleated giant cells and marrow fibrosis. ($\times 250$.)

single case, but those which occur often enough in the spontaneous diseases of man to be of diagnostic importance have been reproduced with great regularity by these experiments.

The confusion of nomenclature in connection with diseases of the bone is particularly apparent in the literature of hyperparathyroidism, and although it was expected that a bone disease would result from such repeated injections of parathormone it could not be foretold whether this would be osteitis fibrosa, osteomalacia or rickets. Erdheim postulated that the enlargement of the parathyroids, seen so often in association with osteomalacia, represented an inadequate attempt at compensation and some clinicians have credited him with the same view for osteitis fibrosa and have used the terms osteomalacia and osteitis fibrosa interchangeably. From the results of these experiments, and others to be reported, in which animals receiving parathormone were simultaneously treated with viosterol, it is evident that the two diseases are distinct and that hyperfunction of the parathyroid glands, while secondary in the case of osteomalacia or rickets, is a primary factor in the pathogenesis of osteitis fibrosa. The relationship of these conditions will be discussed more fully in a subsequent report.

Summary. The experimental production in puppies of a state of chronic hyperparathyroidism is reported and descriptions are given of the gross, roentgenologic and microscopic abnormalities resulting therefrom. This experimental disease, produced with repeated injections of parathormone, is characterized by skeletal lesions and other abnormalities which correspond closely to those observed in clinical cases of osteitis fibrosa osteoplastica (von Recklinghausen).

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EXPERIMENTAL CHRONIC HYPERPARATHYROIDISM.

IV. EFFECTS OF ADMINISTRATION OF IRRADIATED ERGOSTEROL.

BY JOSEPH L. JOHNSON, M.D.,*

CHICAGO, ILL.

(From the Department of Medicine, University of Chicago.)

THE production of chronic experimental hyperparathyroidism in man, rats and dogs has been discussed in three previous papers.^{10,11,12} The skeletal lesions resulting from repeated injections of parathormone are those observed in a disease first described by von Recklinghausen and named by him osteitis fibrosa osteoplastica. The influence of irradiated ergosterol on the course of this experimental disease is the subject of the present communication.

A relationship between the parathyroid glands and vitamin D has been suggested by the observations of a number of investigators. Nonidez and Goodale,¹⁶ and Higgins and Sheard⁸ noted hyperplasia of the parathyroids in fowls deprived of vitamin D. Hess, Lewis and Rivkin⁷ found that irradiated ergosterol no longer raised the level of serum calcium in a parathyroidectomized monkey although the same animal had, previous to operation, responded by a rise to the normal from a serum calcium level previously lowered by a diet deficient in calcium. Similar observations on dogs led them to the conclusion that vitamin D would stimulate parathyroid activity. Prevailing views as to the mechanism of this action are conflicting. Morgan and Garrison¹⁵ agree with Hess that parathyroid activity is more pronounced in the presence of vitamin D, but differ from him by denying that any more hormone is produced. In other words, the action of the vitamin according to them is upon the hormone rather than the glands. Also Jones, Rapoport and Hodes¹⁴ have produced hypercalcemia in parathyroidectomized dogs when large doses of irradiated ergosterol were used and have found that a pre-operative administration of irradiated ergosterol prolongs life, and either prevents or delays the onset of tetany in thyroparathyroidectomized dogs. They are

* The expenses of this research were met from the John D. Hertz Fund.

TABLE 1.—ANALYSIS OF DATA.

Dog or rat No.	Sex.	Days on ex- peri- ment	Parathormone administration.		Vioosterol administration.		Gross observations (b).	Roentgenologic observations.	Microscopic observations.
			Dose in units	Total units	Dose in drops.	Total drops.			
Rat A	F.	10	20 ⁸	160	15 ⁸	120	Marked deformities of fore and hind limbs. ⁶ Blood-tinged feces. ⁴ Muscular weakness and hypotonia. ⁵ Knobbing of wrists; bowing of fore limbs. ⁹	Mottling and rarefaction of all bones; moth-eaten appearance of ends of bones; multiple cysts; deformities; thinning and absence of cortex; visible kidneys; striking changes.	Replacement of bone and marrow by fibrous tissue; osteoporosis and bone absorption; giant cells; striking changes.
Rat 5	F.	43	10 ⁸ (7) 20 ³ 15 ¹⁴ (16) 20 ⁵	430	15 ⁷ 30 ⁸ (13) 60 ¹⁵ (21)	1240	Eating very little; muscular weakness and hypotonia; no other noticeable changes; at autopsy kyphoscoliosis.	Very striking rarefaction; mottling; moth-eaten appearance at ends of long bones; marked kyphoscoliosis; visible kidneys; striking changes.	Marked fibrosis of bone and marrow; osteoporosis; cysts; giant cells; striking changes.
Rat 7	F.	43	10 ²⁷ (29) 15 ⁷	375	15 ¹⁵ (19) 30 ⁸ (26)	750	No appreciable changes.	Rarefaction of skeleton; suggestion of kidney outline.	(Osteoporosis; bone resorption; fibrosis of bone and marrow; cysts; mild changes. Osteoporosis; slight changes.
Rat 15	F.	3	20 ² (3)	40	10 ² (3)	20	No appreciable changes. Found dead. ³	Slight bone destruction at lower end of left femur and upper end of left tibia.	(Osteoporosis; slight changes.
Rat 20	F.	12	15 ⁴ (8)	60	15 ⁷ (11)	105	Red material around eyes resembling dried blood; ⁴ depressed; injections stopped; found dead in cage, body partially eaten by No. 21.	Rarefaction; mutilated skeleton; slight changes.	Slight osteoporosis; slight bone resorption and fibrosis of bone and marrow; slight changes.
Rat 21	F.	33	15 ¹⁴ (19)	210	15 ¹⁷ (23)	255	Red material around eyes resembling dried blood, slight deformity of all feet; ⁹ general condition very good; ²⁹ marked bowing of fore limbs and knobbing of wrists; ³⁰ condition good except for deformities; slight muscular weakness and hypotonia; ²³ treatment interrupted for one week. ³¹ Killed. ³³	Rarefaction; mottling; moth-eaten appearance of ends of long bones; deformities, slipped epiphysis; suggestion of kidney outline; marked healing; striking changes.	Evidence of recovery from fractures; bone destruction and osteoporosis; much callus formation; mild changes; studied after healing had started.

Rat 27	F.	8	15 ³	45	15 ⁷	105	Condition poor. ⁷ Found dead. ⁸	Rarefaction; bone destruction; cysts; visible kidneys: mild changes.	Osteoporosis; resorption; replacement of bone and marrow by fibrous tissue; giant cells; a circumscribed area of fibrous tissue with numerous giant cells; marked changes.
Rat 30	F.	32	15 ³ (9) 10 ¹ (1)	55	15 ³⁴ 15 ⁴ (5)	420	Started as a control; shifted to acterol alone; conditions remained excellent for entire period on acterol alone 24 days; parathormone started; ²⁵ not eating; losing weight. ²⁸ Treatment stopped because of poor condition. ³⁰ Found dead. ³²	Early films showed no changes; film after 4 days on parathormone showed bone destruction, pathologic fracture and visible kidneys: mild changes.	Osteoporosis; bone resorption; marrow necrosis; fibrosis of bone and marrow; cyst formation; mild changes.
Dog 41	F.	58	57 (1) 10 ¹¹ (12) 15 ¹³ (4) 20 ¹⁰ (18)	545	40 ¹¹ (11) 20 ⁴² (48)	1280	Hind legs turn outward at hips; hind legs weak; knobbing of wrists; ⁴⁴ hind legs markedly bowed; plantigrade stance; waddling gait. ⁴⁹ Motion pictures made. ⁵⁷ Skin lesion; both cornea and sclera slate blue color. Killed. ⁵⁹ Autopsy: Very flexible fibula; bones purplish red color flexible; ulna, enlarged ends of bones.	Moth-eaten appearance in places; epiphyseal cartilage widened at lower end of radii, suggestive of rickets; no very striking changes.	Definite fibrous tissue replacement of bone and marrow. Haversian and communicating canals enlarged and filled with fibrous tissue. Numerous giant cells; isolated islands of cartilage; cyst formation; striking changes.
Dog 42	F.	55	57 10 ¹⁶ 20 ²⁷ 15 ¹²	777	40 ¹¹ 20 ⁴⁵	1340	Knobbing of wrists; muscular weakness and hypotonia, genu valgum, bowing of fore limbs. ³⁸ Treatment stopped for 2 days because animal could not stand; crawled on stomach to play with other animals; blood-tinged stools. ⁴⁵ recovered; motion pictures made. ⁴⁸ Killed. ⁵⁷ Autopsy: Fibula very fibrous and flexible; pelvis flexible; small brownish circumscribed area in shaft at lower part of femur.	Curved fibula observed long before killing; fracture of both humeri; bowing; thick cortex; moth-eaten ends of long bones; cysts in pelvis; mild changes.	Pathologic fracture; massive hemorrhage into marrow spaces; striking replacement of bone and marrow by fibrous tissue; giant cells.

Control animals grew normally, remained in excellent condition and showed normal bones roentgenologically and histologically.

(a) The numerals in this column used as exponents and without parentheses represent the number of injections of the specified dose and the numerals within parentheses represent the number of days over which these injections were spread.

(b) The numerals in this column represent the day of the experimental period when the observations were made.

thus agreed with Morgan and Harrison that the action of vitamin D is not upon the glands and regard its effects as supplementary to those of the hormone.

From incompletely controlled observations in a clinical case of osteitis fibrosa with parathyroid gland tumor, the impression was gained that cod-liver oil (vitamin D) was clinically beneficial. Wilder²⁰ pointed out that an antagonism was suggested by this observation, and Compere³ and Quick and Hunsberger¹⁷ have since reported clinical experience which seemed to substantiate this hypothesis of antagonism. However, the interpretation placed upon the experiments of Nonidez and Goodale, and of Higgins and Sheard, namely, that the action of the parathyroids and vitamin D is supplementary, is quite untenable if this is true, because, as Wilder stated, "if irradiated ergosterol supplements the parathyroids its exhibition should aggravate, certainly not benefit, conditions attributable to overfunctioning of the parathyroid glands."

It was for the purpose of obtaining more definite experimental evidence in this problem that irradiated ergosterol was given to animals receiving parathormone. The results obtained have convinced me of the essential difference between either osteomalacia or rickets and osteitis fibrosa. The osteitis produced by chronic injections of parathormone^{9,10,11} was actually aggravated by the simultaneous administration of irradiated ergosterol and no antagonism whatsoever could be demonstrated.

Methods. The study covers experiments with rats, dogs and human subjects. Metabolic investigations formed the most important feature of the observations on man. The methods and results of these have been reported by Johnson and Wilder.¹² In the work with rats and dogs the same methods were used as were reported^{10,11} for experiments with parathormone alone, except that irradiated ergosterol (viosterol) was administered by mouth. Litter mates of the animals which received parathormone alone served for this purpose, the experiments running simultaneously. The same dosage of parathormone was used in both groups of animals. The rats received 10 to 20 units of parathormone daily, and 10 to 15 drops of viosterol.* In the case of Rat 5, 15 to 60 drops were given, and Rat 7, received 15 to 30 drops (Table 1). The puppies had from 2 to 20 units of parathormone daily and from 3 to 40 drops of viosterol. The latter was mixed with the food for the rats and in the case of the puppies was dropped directly into the back part of the mouth. Its introduction always preceded the parathormone injections by several days. Other litter mates on identical diets, but no medication, served as controls.

One rat, No. 30, was carried on viosterol alone for 24 days and then upon parathormone and viosterol for 5 days (Table 1).

Results. Fifteen puppies and 20 rats received viosterol together with parathormone in this series of experiments. Illustrative protocols are presented in the accompanying tabulation. The results in the early studies with puppies suggested some protection from the irradiated ergosterol, but later experiments with puppies and rats,

* A dropper furnished with the Johnson-Mead product was always used.

and the metabolic data obtained with human subjects, disprove this and clearly indicate that irradiated ergosterol not only is not antagonistic in its action but actually intensifies the effects of para-



FIG. 1.—Dog 42. Bowing of fore legs after parathormone and viosterol administration of 38 days. Animal received from 5 to 20 units of parathormone daily during this period. For first 11 days of experiment 40 drops of viosterol was given each day after which dose was reduced to 20 drops daily.

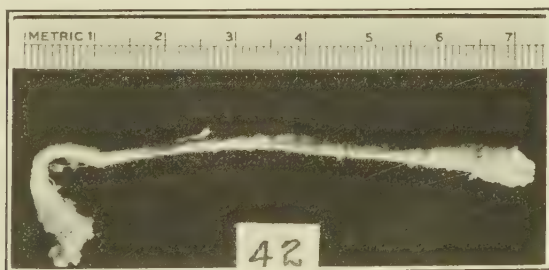


Fig. 2.—Dog 42. Flexible and fibrous right fibula after parathormone and viosterol administration. Animal killed to terminate experiment on 67th day. In 52 days 777 units of parathormone was administered in doses of from 5 to 20 units a day. 1340 drops of viosterol was given in 56 days, 40 drops a day for first 11 days and 20 drops for 45 days.

thyroid hormone. The same outstanding lesions, observed by gross, roentgenologic and microscopic examinations in the parathormone experiments^{10,11} were equally and usually more prominent in animals treated with both parathormone and irradiated ergosterol. (Figs. 1 to 12.)

Among the most interesting additional observations in the parathormone viosterol experiments was a marked metastatic calcification of the kidneys. In no instance, when parathormone was given alone, could the kidney be definitely visualized by roentgenograms, although in 2 cases in rats there were faint outlines suggestive of such visualization. With the simultaneous administration of parathormone and viosterol the kidney of the rat was repeatedly and clearly visible in the roentgenograms. (Fig. 3.)

In order to answer the question as to whether or not irradiated ergosterol was alone responsible for this greater degree of metastatic calcification, Rat 30 was kept on this vitamin D concentrate for 24 days, before being given parathormone. At the end of that time no metastatic calcification was evident roentgenologically (Figs. 6 and 7), and on the twenty-fifth day 15 units of parathormone was injected. This dose was continued for 3 days, whereupon the previously excellent condition of the animal changed to a poor one. The dose was then decreased to 10 units for 1 day and discontinued. In roentgenograms made when this treatment was stopped the kidneys are clearly visible. (Fig. 8.) The animal died 2 days later.

The roentgenograms of Rat 21 bring out another interesting feature, namely, the rapidity of healing of the skeletal lesion. This animal had developed all of the gross deformities and roentgenographic changes of osteitis fibrosa. (Fig. 4.) With other conditions remaining the same, parathormone and viosterol were discontinued. Seven days later roentgenograms revealed a very striking amount of healing. (Fig. 5.)

The histologic examinations of the bones of both the rats and dogs in these experiments revealed extensive resorption of trabecular and cortical bone, proliferation of osteoid tissue, cyst formation, giant cells and replacement of bone and marrow by fibrous connective tissue. (Figs. 9, 10 and 11.) The kidneys showed enormous calcium deposits in the tubules and interstitial tissue but no stone formation. (Fig. 12.)

Discussion.—A comparison of the results in these experiments with those reported previously^{10,11} shows that the skeletal abnormalities were as great and usually greater when irradiated ergosterol accompanied the injections of parathormone. Also, as reported earlier,¹⁰ the pain, muscular weakness, headaches and lassitude experienced by the human subject were worse during the period of parathormone and irradiated ergosterol than they had been during the parathormone period. The negative calcium and phosphorus balances of the parathormone period of the human experiment became greater during parathormone and irradiated ergosterol administration.¹² These observations indicate that irradiated ergosterol actually aggravates the conditions attributable to hyperparathyroidism.

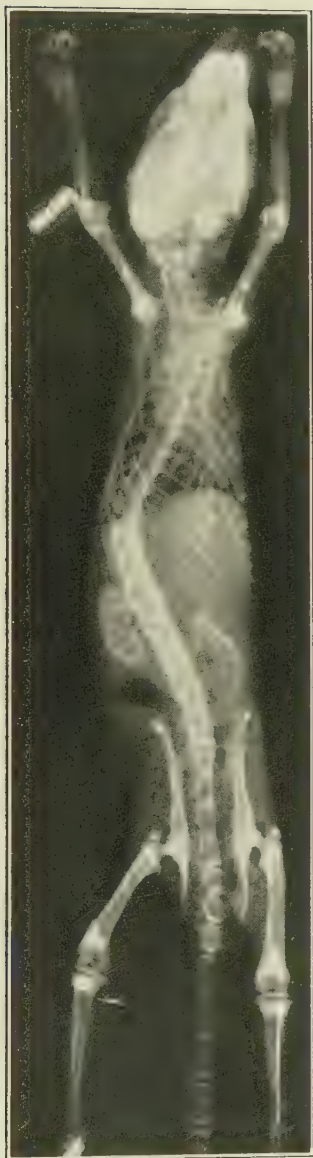


FIG. 3.

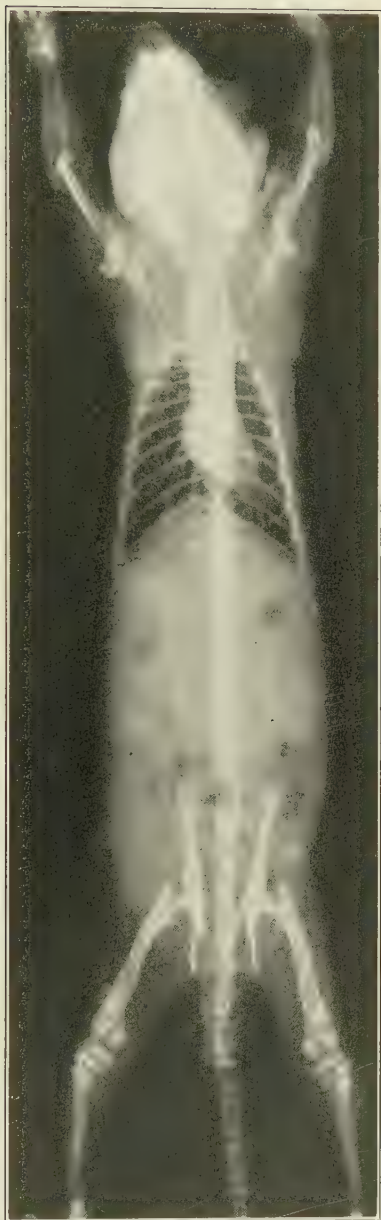


FIG. 4.

FIG. 3.—Rat 5. Rarefaction and resorption of long bones, kyphoscoliosis and calcified kidneys. Roentgenogram made at termination of experiment on 42d day. A total of 430 units of parathormone had been administered in 31 days in doses of from 10 to 20 units per day. 1240 drops of viosterol was given in 51 days in quantities of from 15 to 60 drops a day.

FIG. 4.—Rat 21. Marked bone destruction at upper end of each tibia and slipped epiphysis at head of humerus. Roentgenogram made on 26th day of experiment. A total of 210 units of parathormone in doses of 15 units a day and a total of 255 drops of viosterol at rate of 15 drops a day had been administered. Fig. 5 shows healing in same animal 7 days later.



FIG. 5.

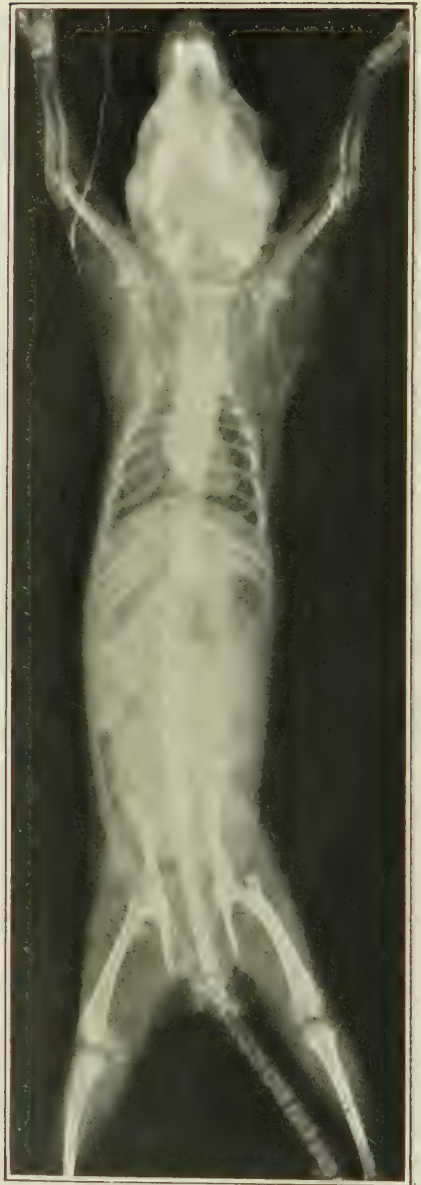


FIG. 6.

FIG. 5.—Rat 21. Healing at the upper end of each tibial after medication had been stopped for 7 days. Fig. 4 shows the condition before medication was stopped.

FIG. 6.—Rat 30. Animal used to determine whether or not viosterol alone would cause calcification of kidneys to extent of roentgenologic visualization. 15 drops of viosterol per day was given for 24 days. No evidence of bone lesions or calcification of kidneys after 8 days on viosterol. (In series with Figs. 7 and 8.)



FIG. 7.



FIG. 8.

FIG. 7.—Rat 30. No evidence of bone lesions or kidney calcification after 24 days on viosterol. (Roentgenogram made 16 days after Fig. 6.)

FIG. 8.—Rat 30. Rarefaction of skeleton bone destruction and striking calcifications of the kidneys. This roentgenogram was made 7 days after that shown in Fig. 7. During the first 4 of these 7 days the animal received 15 units of parathormone daily in addition to viosterol.

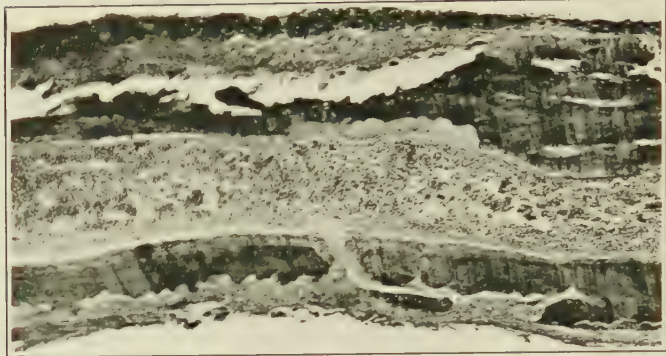


FIG. 9.—Rat A. Resorption of cortical bone. ($\times 60$.) Animal died on 10th day of experiment and had received 20 units of parathormone daily for 8 days and 15 drops of viosterol a day for 8 days.

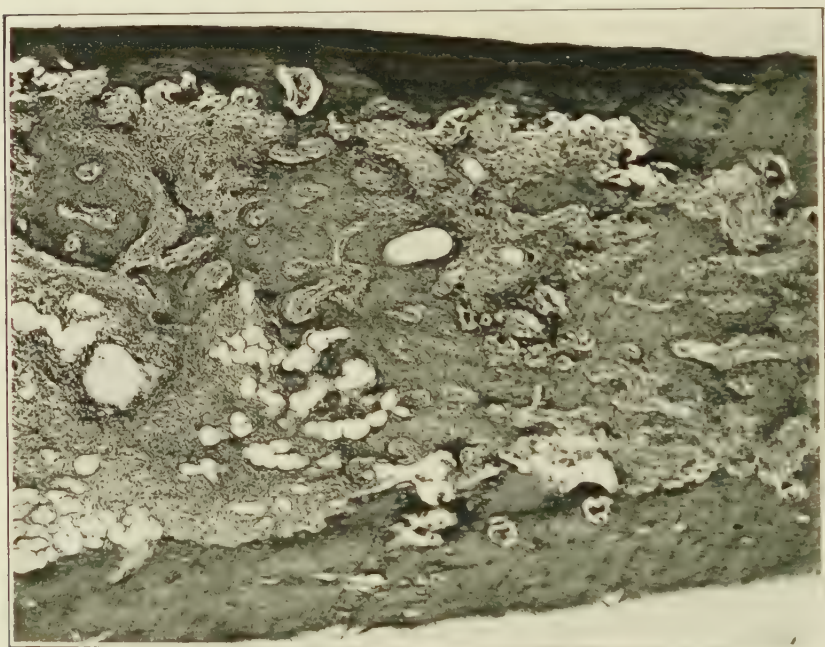


FIG. 10.—Rat A. Fibrous tissue replacement of bone and bone marrow, lacunar resorption, cysts, osteoid tissue and giant cells. ($\times 60$.) See legend of Fig. 9.



FIG. 11.—Rat 5. Fibrous tissue replacement of bone, filling in of enlarged canals of the cortex by fibrous tissue, cysts and osteoid tissue. ($\times 30$.) See legend of Fig. 3.

Metastatic calcification has been frequently reported in conditions of parathyroid overfunction, and likewise in connection with the administration of irradiated ergosterol. The kidney in each instance has been one of the most frequent tissues affected. I have found, however, no clinical or experimental reports of renal calcification sufficiently intense to render the kidney visible by roentgenogram. In these experiments neither parathormone nor irradiated ergosterol alone produced sufficient calcification for kidney visualization, but when the two substances were administered simultaneously the kidneys could be clearly seen. (Fig. 3.) On microscopic examination calcium was found in the tubules and to a less extent in the interstitial tissue in the parathormone as well as the parathor-

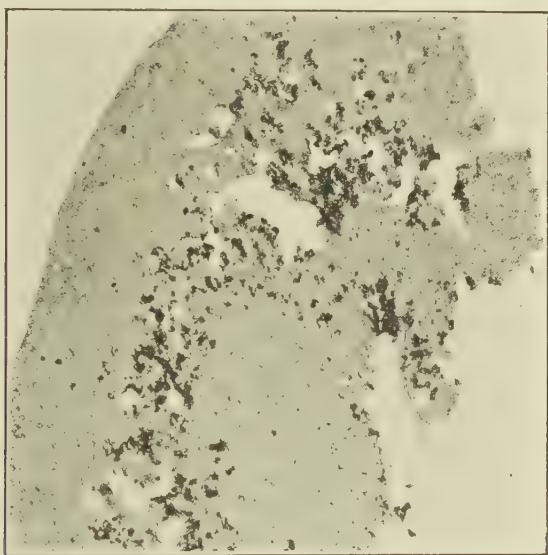


FIG. 12.—Rat 5. Calcium deposits in kidney. ($\times 12$.)

mone irradiated ergosterol animals, but much more abundantly in the latter. (Fig. 12.) There was also considerable damage to renal parenchyma. Nephritis and nephrolithiasis, seen clinically in association with tumor of the parathyroid gland and osteitis fibrosa, may well be explained on the basis of this metastatic calcification.

These experiments permit of the following interpretation. Vitamin D either stimulates the parathyroid glands to secrete more hormone or intensifies the activity of the hormone in circulation or supplements in its action the effect of the hormone. They certainly bring no evidence of inhibition of the glands or neutralization of the hormone, and consequently no support for the clinical treatment

of states of hyperparathyroidism with cod-liver oil, ultraviolet light or irradiated ergosterol. The danger of intensifying metastatic calcification is very real, and for this, if for no other reason, treatment with vitamin D is definitely contraindicated in osteitis fibrosa.

The outcome of the study also indicates the complete etiologic dissimilarity between the osteitis fibrosa and rickets or osteomalacia. The skeletal lesions of the former are intensified by vitamin D concentrates which are so beneficial in the latter. The hypertrophy of the parathyroids that accompanies rickets, as noted first by Erdheim, and the similar hypertrophy of these glands that occurs in fowls that are grown under amber light or are otherwise deprived of vitamin D is a phenomenon secondary to vitamin D deprivation. It may be an attempt at compensation, as originally suggested by Erdheim. That such gland produces more hormone than normal is not improbable, but that this increased hormone output is not in excess of the tissue demand is shown by the absence of lesions like those which appear when parathormone is administered to normal animals. In osteitis fibrosa we have to deal with normal demand and excessive supply of hormone, for which a primary enlargement of parathyroids is responsible. These conclusions are in harmony with the observation that in rickets and osteomalacia hypertrophy involves all of the parathyroids with more or less uniformity, whereas it is a rule in osteitis fibrosa for one or at most two glands to be enlarged as tumors with the remainder remaining normal in size and structure. The case of osteitis fibrosa studied by Hannon and his colleagues,⁶ and later operated upon by Richardson,¹⁸ is no exception to this rule. A careful surgical exploration of the neck failed to reveal either a tumor or any general hypertrophy of the parathyroids, but this only suggests that overfunction of parathyroids and excessive hormone supply may occur in the absence of anatomic abnormalities. Several analogies can be cited, particularly the healthy appearance of the islets of Langerhans in certain outstanding instances of hyperinsulinism, as reported by Allan¹ and the Finneys.⁴

Summary. An answer was sought to the question as to the relationship of osteitis fibrosa to those other skeletal diseases, especially rickets and osteomalacia, that are also associated with hypertrophy of parathyroid glands. Metabolic studies of the problem have been reported previously; the results of animal experiments are given here. Irradiated ergosterol, in a dosage of 5 to 60 drops daily, was fed to young, white rats and puppies maintained in a chronic state of hyperparathyroidism by the daily injection of parathormone. These animals were litter mates of others that developed the skeletal lesions of osteitis fibrosa when treated with parathormone alone.^{10,11} The technique of experimentation, the diets and dosage of the parathormone was the same as that previously reported.^{10,11} The outcome was the same, except that the result-

ing lesions typical of the skeletal abnormalities of osteitis fibrosa were, on the whole, more extensive. Metastatic calcification in the kidneys was also more marked.

Whereas vitamin D concentrates effectively protect against rickets or osteomalacia, it is evident from these experiments that they intensify the disease produced by excess of parathyroid hormone. It is, therefore, clear that rickets or osteomalacia differ essentially in pathogenesis from osteitis fibrosa. While the hypertrophy of the parathyroids in conditions of vitamin D deficiency may be compensatory and is certainly a secondary phenomenon, the tumors of parathyroids found in association with osteitis fibrosa are of primary significance. It is recognized, however, that overfunction of parathyroids may occur in the absence of tumors or other morphologic abnormality.

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